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Review

Going against the flow: A case for peroxisomal protein export

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ABSTRACT

Peroxisomes play a crucial role in regulating cellular metabolism, providing compartments where metabolic pathways can be contained and controlled. Their importance is underlined by the developmental brain disorders caused by peroxisome malfunction, while disturbances in peroxisome function also contribute to ageing. As peroxisomes do not contain DNA, they rely on an active transport system to obtain the full quota of proteins required for function. Organelle protein transport however, is rarely a one-way process and exciting recent data have demonstrated that peroxisomes can selectively export membrane and matrix proteins to fulfil specific functions. This review will summarise the current knowledge on peroxisomal membrane and matrix protein export, discussing the mechanisms underlying export as well as the role of peroxisomal protein export in peroxisomal and cellular function.

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1. Introduction

Peroxisomes play an essential role in cellular metabolism, providing compartments where enzymatic pathways are contained and controlled [1,2]. Peroxisomes are highly versatile, as demonstrated by the wide range of metabolic pathways they contain. Some well-known functions of peroxisomes include the oxidation of fatty acids in many different organisms [3], the biosynthesis of plasmalogens in mammals [4] and penicillin in fungi [5], glycolysis in trypanosomes [6] and the glyoxylate cycle and photorespiration in plants [7]. The importance of peroxisomes in cell vitality and human disease is underlined by the developmental brain disorders caused by defects in peroxisome function [8,9]. Additionally, malfunctioning peroxisomes contribute to ageing [10].

Parcelling processes into organelles presents many advantages for a eukaryotic cell. However it also creates challenges, the largest being regulating transport of molecules in and out of the organelle. All peroxisomal proteins are made in the cytosol [3] and post-translationally imported into peroxisomes with the aid of a peroxisomal targeting signal (PTS) sequence [3]. Proteins destined for the peroxisomal matrix contain one of two signals, a C-terminal PTS1 sequence or an N-terminal PTS2. Peroxisomal membrane proteins (PMPs), on the other hand, possess a membrane PTS (mPTS) sequence, usually present in one of the proteins transmembrane domains [11]. After synthesis, PTS containing matrix

proteins are recognised in the cytosol by specific cycling receptor proteins, which direct their import into peroxisomes [12]. Pex5p functions as the specific receptor for PTS1 containing proteins [13,14], whereas PTS2 proteins are imported by Pex7p, together with an additional co-receptor protein [15]. In yeast, this function is performed by members of the Pex20 protein family [16] while a specialised isoform of Pex5p is responsible in higher eukaryotes [17]. Pex19p is thought to act as cycling receptor for proteins with an mPTS [11,18], although the mechanisms of PMP targeting and import are currently under debate [19].

Organelle protein transport is rarely a one-way process and the protein export pathways of several organelles have been studied in depth. One well-known example is endoplasmic reticulum (ER) associated degradation, known as ERAD. This pathway targets misfolded ER membrane and matrix proteins for degradation in the cytosol [20]. When ERAD becomes blocked, misfolded proteins aggregate, ultimately leading to disease [21]. As with ERAD, failure of mitochondria associated degradation (MAD), a pathway that exports damaged mitochondrial proteins for degradation in the cytosol, contributes to neurodegenerative disease [22]. These examples demonstrate how critical protein export is for organelle function.

The vital role peroxisomes play in cellular metabolism demands an ability to adapt protein content to changes in metabolic needs. For this reason, a pathway to selectively export proteins, for degradation or targeting to other cellular compartments, would be an invaluable asset, particularly when the alternative, wholesale destruction of peroxisomes via autophagy, may not be feasible or desirable. With this in mind, it is surprising that until relatively recently, peroxisomal protein export has received very little attention. In this review, I shall sum up the current knowledge on peroxisomal membrane and matrix protein export, discussing known export events, as well as potential mechanisms and functions. This will also incorporate a number of observations

Abbreviations: PTS, Peroxisomal targeting signal; PMP, Peroxisomal membrane protein; ER, Endoplasmic reticulum; ERAD, Endoplasmic reticulum associated degradation; MAD, Mitochondrial associated degradation; PexAD, Peroxisome-associated matrix protein degradation; UPS, Ubiquitin proteasome system; E2, Ubiquitin conjugating enzyme; E3, Ubiquitin ligase; ICL, Isocitrate lyase; MLS, Malate synthase; INP, Inheritance of peroxisomes; PEST, Proline, glutamic acid, serine, threonine rich region

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that although not described as such, are reminiscent of peroxisomal protein export. Finally, I shall comment on potential future directions and shall also outline a number of important questions that remain to be answered.

2. Lessons from the PTS receptor export process

As mentioned, peroxisomal protein export is a relatively unexplored field. However, one protein export event is well established: removal of the PTS (co-) receptors Pex5p and members of the Pex20p family from the peroxisomal membrane [23,24]. After delivering their cargo to the peroxisome, the PTS receptors are modified by the small protein ubiquitin, facilitating their removal from the membrane [25–28]. In yeast, the ubiquitination of the PTS receptors promotes two different outcomes, depending on the ubiquitin-conjugating enzyme (UBC or E2) involved; recycling to the cytosol, allowing the receptors to take part in further rounds of import or alternatively, degradation by the ubiquitin-proteasome system (UPS), the major protein degradation pathway in eukaryotes [23,29]. Recycling of the (co-) receptor is controlled by Pex4p, together with its co-activator Pex22p [30–32], while receptor degradation requires Ubc4p [25,33]. In mammals, members of the E2D family of E2s regulate both the recycling and probably also degradation of Pex5p [28,34]. Furthermore, a complex of Pex2p, Pex10p and Pex12p, three ubiquitin ligases (E3s) present at the peroxisomal membrane, facilitates ubiquitin attachment [27,35–38]. Finally, removal of the ubiquitinated (co-) receptors from the peroxisomal membrane requires the action of two AAA (ATPases Associated with diverse cellular Activities) proteins, Pex1p and Pex6p [29,39]. In the case that receptor export is for recycling, the additional action of a deubiquitinating enzyme is required, to remove ubiquitin before the receptor can feed back into the matrix protein import cycle [40,41].

Although this review will not cover PTS receptor export further, as this has been well described already [42,43], we can draw a number of interesting comparisons with this pathway, since some of the players appear to regulate the export of other proteins out of peroxisomes. Furthermore, by taking a look at receptor export, we gain two valuable pieces of information: that ubiquitination can control the export of proteins from peroxisomes *and* that export can lead to targeting of the protein to other parts of the cell, or to protein degradation.

3. Presenting the case for peroxisomal protein export

3.1. Peroxisome-associated matrix protein degradation in plants

The first indication that peroxisomes might export matrix proteins stems from a report on cucumber peroxisomes, dating back to 1985 [44]. During seed germination, peroxisomes house the enzymes required for glyoxysomal metabolism and the β -oxidation of fatty acids, processes that are required for germination and the early stages of growth. However, after around 4 days, once the seedlings are able to grow photoautotrophically, these enzymes are no longer needed and peroxisomes instead function in the photo respiratory pathway. At the time, two theories existed to explain this apparent change in function, referred to respectively as the one- or two-population models [45,46]. The one-population model suggested that peroxisomes rearranged their protein content to meet the metabolic requirements of the plant, whereas the two-population model predicted the existence of two types of peroxisome, which contain enzymes for either glyoxysomal metabolism or photorespiration. The peroxisomes that house glyoxysomal enzymes are degraded and replaced by peroxisomes housing enzymes of the photo respiratory pathway. By following peroxisomes with electron microscopy [44], Titus and Becker revealed that isocitrate lyase (ICL) and malate synthase (MLS), two proteins of the glyoxylate cycle, were present in peroxisomes at around 2 days post-germination yet these proteins were absent from 8-day-old peroxisomes. Instead, 8-day-old peroxisomes contained enzymes of the photo respiratory

pathway, such as hydroxypyruvate reductase and serine:glyoxylate aminotransferase. Importantly, both sets of enzymes were found in peroxisomes from 4-day-old seedlings.

These observations supported the idea that obsolete enzymes from the glyoxylate cycle were specifically removed from peroxisomes and degraded in a development-coordinated manner. A number of questions remained however, including *where* this degradation event occurred, in the peroxisome or in the cytosol, and which quality control system was responsible, peroxisomal proteases or the cytosolic proteasome? In-depth insights into the mechanisms behind ICL and MLS turnover come from a series of publications in *Arabidopsis* [47–50]. First, Zolman et al. demonstrated that the E2 Pex4p, together with its co-activator Pex22p, was required for ICL degradation, suggesting not only that the ubiquitin cascade could be involved, but also that proteins which control matrix protein import regulate matrix protein degradation [50]. Next Lingard et al. revealed that ICL and MLS degradation was inhibited in cells disrupted for the import receptor Pex5p or the ATPase Pex6p [49]. Significantly, the enzymes were stabilised in the cytosol in the *pex5* mutant but in peroxisomes in the *pex6*, indicating two central points; that import is a pre-requisite for degradation *and* that the degradation event does not occur inside peroxisomes. These observations lead the authors to propose the existence of a ubiquitination regulated, retro-translocation pathway to remove obsolete proteins from the peroxisomal matrix, which they termed the “Peroxisome-associated matrix protein degradation” (PexAD) pathway (Fig. 1, adapted from [47]). Support for this proposal comes from a large-scale proteomics approach, which demonstrates that ICL (along with a number of other peroxisomal matrix proteins) is indeed ubiquitinated in plant cells [51]. Furthermore, an additional report from the Bartel group identified a mutant form of Pex6p that specifically inhibited ICL and MLS degradation yet was virtually unaffected in other peroxisomal processes, implying that separate

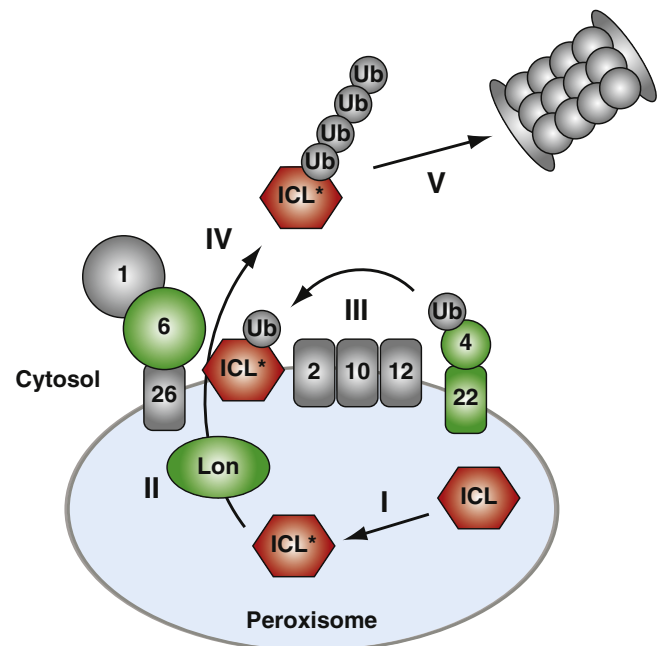


Fig. 1. Peroxisome-associated matrix protein degradation in *Arabidopsis*. Model describing how obsolete isocitrate lyase (ICL, depicted in red) may be exported out of peroxisomes (adapted from [47]). Influenced by peroxisomal metabolism and/or possibly hydrogen peroxide damage (I), Lon protease facilitates targeting of ICL to the export machinery (II). Next, ICL ubiquitination is mediated by the E2 Pex4p, probably with the aid of the E3 ligases Pex2p, Pex10p and Pex12p present on the peroxisomal membrane (III). Ubiquitinated ICL is then recognised by Pex6p and exported to the cytosol (IV), where degradation occurs, most likely through the action of the proteasome (V). Individual Pex proteins are numbered. Defects in the proteins shown in green are known to inhibit ICL degradation.

regions of Pex6p control matrix protein import and ICL/MLS degradation [47].

When taken together, all these observations convincingly make a case for peroxisomal matrix protein export. However, the latest report from Farmer et al. [48] has muddled the waters slightly. This publication demonstrated that mutants disturbed in autophagy exhibited a slightly slower MLS turnover rate, hinting that MLS degradation in wild type cells may not exclusively occur via the PexAD pathway. In addition, they identified a role for the peroxisomal Lon protease in ICL/MLS turnover. The ATP dependent Lon protease is a known contributor to peroxisomal quality control events and was previously shown to degrade misfolded or oxidised substrates inside peroxisomes [52,53]. Furthermore, in higher eukaryotes, the action of Lon protease is required for matrix protein import [54,55]. Could this suggest that ICL/MLS degradation is actually fulfilled by a peroxisomal protease rather than by a retro-translocation pathway? While such an eventuality cannot be excluded, because of the known role Lon protease plays in quality control, the vast majority of the data point towards ICL/MLS degradation being a cytosolic event, and one that first requires these proteins to be imported into peroxisomes. Where could Lon then fit into this pathway? Lon protease from the filamentous fungus *P. chrysogenum* possesses chaperone activity [53], which may indicate its role is to recognise substrates and to chaperone them to the export machinery. Time will tell the full extent to which Lon regulates the PexAD pathway.

3.2. Ubiquitination controls export of the PMP Pex3p in *H. polymorpha*

As with matrix protein export, the first report on peroxisomes exporting PMPs concerned protein degradation [56]. Peroxisomes proliferate in response to a number of internal or external stimuli. This is particularly evident in yeast, where peroxisome proliferation is induced during growth on specific carbon or nitrogen sources [57]. On the contrary, peroxisomes can be swiftly degraded by pexophagy when they become redundant for growth, again demonstrating their agility in adapting to changes in the metabolic requirements of the cell [58]. Peroxisome proliferation in the methylotrophic yeast *H. polymorpha* is induced by methanol, whereas peroxisomes are degraded by the vacuole when methanol-grown cells are exposed to glucose [59]. Bellu et al. demonstrated that initiation of pexophagy required the PMP Pex3p to be removed from the peroxisomal membrane and degraded [56]. Pex3p plays a central role in peroxisome formation [19] and is thought to function as a docking factor for Pex19p, the cycling receptor that transports newly synthesised PMPs to peroxisomes. Intriguingly, Pex3p degradation even occurred when vacuole function was inhibited [56]. While at the time the underlying mechanisms of pexophagy-induced Pex3p degradation were not known, a hint came from the observation that the proteasome inhibitor MG132 interfered with Pex3p degradation and, consequently, pexophagy [56]. It is interesting to note that MG132 also blocks the turnover of Pex3p in mammalian cells [60], suggesting that Pex3p degradation is conserved from yeast to man.

Involvement of the proteasome in Pex3p degradation implies a role for ubiquitin and indeed, Pex3p is ubiquitinated after cells were shifted from methanol to glucose [61]. Ubiquitinated Pex3p peptides were also identified in a large-scale proteomics study on mammalian cells, again providing a link between Pex3p turnover in yeast and man [62]. Similar to the effect of MG132, inhibiting the ubiquitination of Pex3p, through introduction of lysine to arginine mutations into Pex3p or ubiquitin, also blocks glucose-induced pexophagy. Furthermore, Pex3p degradation requires Pex2p and Pex10p [61], peroxisomal E3 ligases shown to regulate ubiquitination of the cycling receptors [27,35–38], implying that the role these proteins play in peroxisome function goes much deeper than only to ubiquitinate the cycling receptors (Fig. 2).

One intriguing observation from this work is that the ATPase Pex1p appears not to be involved in Pex3p degradation [61]. Due to the well-established role of Pex1p in exporting the cycling receptors from the

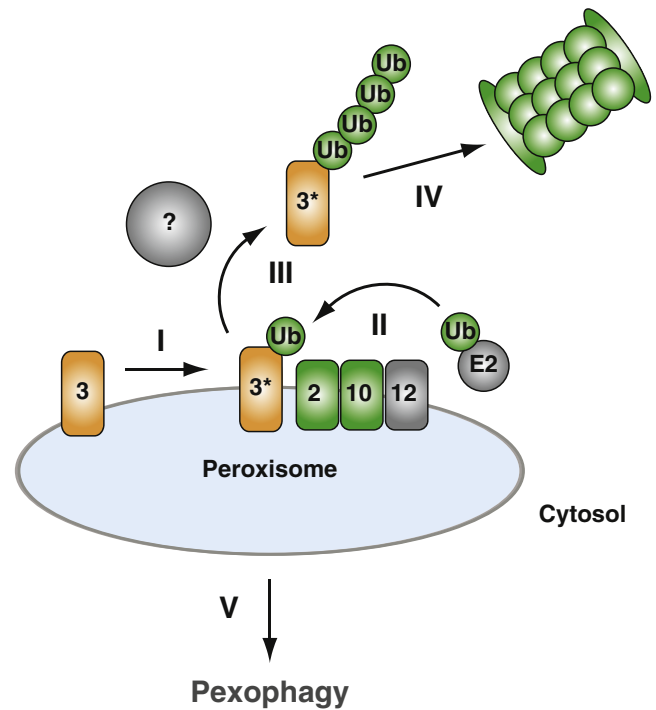


Fig. 2. Model of Pex3p degradation in *H. polymorpha*. Addition of glucose to methanol-grown cells primes Pex3p (orange) for export (I). Pex3p ubiquitination (II) is controlled by an unknown E2, with the aid of the peroxisomal E3 ligases. Modified Pex3p is then removed in a Pex1p-independent manner (III) and targeted to the proteasome for degradation (IV). Upon Pex3p removal, the peroxisome is selectively degraded via pexophagy (V). Individual Pex proteins are numbered. Inhibiting the function of the proteins shown in green blocks Pex3p degradation, and hence pexophagy.

peroxisomal membrane [29,39], it could be expected that Pex1p may have a hand in removing ubiquitinated substrates from the peroxisomal membrane. The same could be said for its binding partner Pex6p, not only because of its role in receptor export, but also due to its involvement in matrix protein export [47]. While a role for Pex6p in Pex3p degradation was not investigated in this work, the requirement of the Pex1p–Pex6p complex for functional export of the cycling receptors would perhaps argue against its involvement. This could indicate a certain degree of substrate specificity prevails in the export pathway, although it is too early to say whether different proteins control the membrane dissociation event for different substrates.

3.3. Further evidence for protein export from peroxisomes

As already mentioned, there are a number of observations in the literature that, when observed in the context of the reports discussed above, very likely represent peroxisomal protein export events.

In the same study that demonstrated Pex3p turnover occurs in mammalian cells, the authors also examined the stability of additional peroxisomal proteins, reporting that the PMP Pex16p was, like Pex3p, stabilised by the presence of MG132 [60]. Not much is known about Pex16p, other than it plays a role in the recruitment of other PMPs to peroxisomes in mammals and that it seems to route to peroxisomes via the ER [18,63], which makes predicting a role for Pex16p export in peroxisome function difficult.

During cell division, cells control the partition of organelles to the mother and daughter cell, to guarantee that both receive their required quota of organelles. Two proteins in yeast, termed inheritance of peroxisomes protein (Inp) 1 and 2, have important but opposite functions during organelle partition [64]. Overexpression of Inp1 causes an increase in the number of daughter cells without peroxisomes, due to its function in

anchoring peroxisomes in the mother [65]. Deletion of INP2 also causes the mother cells to retain peroxisomes, since Inp2 is needed to transport peroxisomes to the daughter cell in a myosin-dependent manner [66]. Therefore, a fine balance between Inp1 and Inp2 is required to ensure the correct partitioning of organelles between mother and daughter. By subjecting *S. cerevisiae* cells to and releasing them from G1-arrest, Fagarasanu et al. demonstrated that both Inp1 and Inp2 levels increase and decrease in a cell cycle dependent manner, which suggests that the levels of these proteins are actively regulated [65,66]. Although the authors did not investigate this further, they did identify a PEST sequence in Inp1. PEST sequences are regions of proteins rich in proline, glutamic acid, serine and threonine residues that target short-lived proteins for degradation, either via the UPS or the vacuole [67]. Whether PEST sequences play a role in Inp1, or indeed in other PMP degradation events remains to be determined.

Peroxisomes contain a number of proteins whose role is to counter the reactive oxygen species produced by the enzymatic pathways contained within the organelle. One such protein is yeast *PMP20*, a member of the peroxiredoxin protein family [68,69]. Peroxiredoxins are involved in the degradation of hydrogen peroxide and organic hydroperoxides. Deletion of *PMP20* in *H. polymorpha* results in increased levels of oxidative stress when cells were grown on methanol-containing media, ultimately leading to necrotic cell death [70]. However, peroxisomes from *pmp20Δ* cells also “leak” peroxisomal matrix proteins to the cytosol under the same conditions. This was observed with green fluorescent protein fused to a PTS1 sequence and with catalase although not, significantly, with all peroxisomal matrix proteins. Subsequent electron microscopic analysis demonstrated that this leakage was not due to ruptured peroxisomal membranes or to pexophagy, indicating that a level of regulation was present in the process. While the mechanisms behind this “leakage” remain unknown, the authors made an interesting comparison to the targeting of cytochrome c from mitochondria to the cytosol and nucleus during the early stages of apoptosis [71]. Cytochrome c is a soluble protein associated with the inner membrane of the mitochondria that, upon export from mitochondria, recruits and activates several factors that trigger apoptosis [72]. The authors suggested that, in cells lacking *PMP20*, matrix proteins target to other cellular compartments and initiate the necrotic cell death response seen in this strain. Should this be the case, it would demonstrate that peroxisomal matrix protein export, as with the cycling receptors, is not exclusively for protein degradation.

Based on some of the data presented above, it is evident that ubiquitination plays an important role in the export of at least a sub-set of peroxisomal proteins, suggesting that ubiquitinated peroxisomal proteins are potential export substrates. Large-scale proteomic approaches in human, yeast and plant cells have provided us with a huge list of potential targets of ubiquitination [51,62,73–75], including several peroxisomal membrane and matrix proteins. Amongst others, ubiquitinated peptides of the PMP Pex14p were found in *S. cerevisiae* [73–75] and human cells [62]. Pex14p plays an important role in allowing the cargo-carrying cycling receptor proteins to dock on the peroxisomal membrane and is thought to represent the first site of contact for this receptor-cargo complex on the membrane [76–78]. Others have gone further, suggesting that Pex14p also facilitates cargo insertion into peroxisomes [79,80]. Adding weight to the suggestion that Pex14p could be exported, several reports in the yeast *H. polymorpha* are worthy of note. In cells deleted for *PMP20*, levels of unphosphorylated Pex14p are rapidly reduced in response to exposure to methanol, a phenomenon that is not observed with the phosphorylated form of Pex14p, or with the PMP Pex11p [70]. In addition, Pex14p levels appear increased in cells deleted for one of the peroxisomal E3 ligases Pex2p, Pex10p or Pex12p [81] or for the E2 enzyme Pex4p [82]. Based on these data alone, it is possible only to speculate on the role ubiquitination of Pex14p may play in peroxisome function and further data on Pex14p ubiquitination are eagerly awaited.

Finally, a recent report from the Aitchison lab provides some interesting food for thought [83]. Employing a systems biology-based

proteomics approach, this group followed condition-specific differences in the abundance and cellular localisation of *S. cerevisiae* proteins, comparing cells grown on glucose to those grown on oleate. This work, which elegantly demonstrates the huge response cells make when adapting to changes in metabolic requirements, grouped together proteins that exhibited similar profiles i.e. up or down regulation in response to oleate, changes in cellular localisation, and so forth. The most interesting group (termed Group V, [83]), at least for this particular review, contains 950 proteins that appeared to dissociate from organelles (mitochondria, peroxisomes, Golgi and the ER) and target to other parts of the cell in response to the presence of oleate. Whether any of these dissociation events represent peroxisomal protein export remains to be determined.

4. Peroxisomal protein export: quality control or targeted degradation?

ICL, MLS and Pex3p all undergo export for protein degradation. Does this mean that peroxisomal protein export serves a quality control function, as do the ERAD and MAD pathways for, respectively, the ER and mitochondria? Two main quality control pathways are known to regulate peroxisome function. The first involves the action of peroxisomal proteases, such as the previously mentioned peroxisomal Lon protease [51,52] or insulin degrading enzyme [84], which degrade damaged proteins inside peroxisomes. Pexophagy on the other hand, the second major quality control mechanism, targets whole peroxisomes for destruction in the vacuole [58]. Peroxisomal protein export does, it seems, contribute to peroxisomal quality control. The turnover of ICL and MLS in plants occurs more rapidly when hydrogen peroxide levels are increased, whereas the opposite is true when hydrogen peroxide production is inhibited [49]. Since peroxisomes are one of the biggest producers of oxidative stress in the cell [85], the presence of an export pathway for the removal of damaged peroxisomal proteins, matching the ERAD and MAD pathways, would not be out of place. Indeed, the reduction of Pex14p levels in cells deleted for *PMP20* may corroborate such a theory [70]. However, pexophagy-induced degradation of Pex3p, regulation of Inp1p and Inp2p in a cell cycle dependent manner and degradation of ICL/MLS under normal conditions are not cases of damaged proteins being removed but instead correspond to the targeted degradation of specific proteins.

Pex3p degradation in *H. polymorpha* triggers pexophagy, therefore acting as a signal to control the peroxisomal and cellular response to changes in metabolic needs. Inp1/2 turnover is finely tuned to the cell cycle, a situation that allows peroxisome inheritance to occur at precisely the right moment. With these data in mind, interesting parallels can be drawn with the targeted down-regulation of the ER membrane proteins Cyclooxygenase 2 and HMG-CoA reductase by the ERAD pathway [20]. Cyclooxygenase 2, which is a key enzyme involved in prostaglandin biosynthesis, contains a C-terminal sequence that targets the protein for ERAD mediated degradation [86]. This mechanism is believed to keep Cyclooxygenase 2 protein levels in check, since excessive Cyclooxygenase 2 levels are known to result in uncontrolled inflammatory responses [87]. HMG-CoA reductase is the rate limiting enzyme involved in the biosynthesis of cholesterol, as well as nonsterol isoprenoids [88]. When levels of sterols in the ER membrane become too high, a feedback mechanism facilitates the selective ubiquitination and degradation of obsolete HMG-CoA reductase via ERAD [89].

These examples provide an extremely interesting insight into how targeted protein export can control specific cellular events. Pex3p and Pex16p levels are significantly higher in mammalian cells treated with the proteasome inhibitor MG132 [60]. Since further information on the turnover of these proteins in mammals is lacking, we can only guess at the role export could play in peroxisome function. However, it is tempting to speculate that Pex3p/Pex16p turnover in mammalian cells could represent a control mechanism to shutdown import of PMPs to peroxisomes. In support of this idea, excessive Pex3p levels

are known to inhibit peroxisome function in several organisms, suggesting that regulatory mechanisms may be required to keep protein levels in check [60,90,91]. On the contrary, these events may stem from the export pathway targeting damaged proteins for removal and degradation. Further data on these events are eagerly awaited to determine how they influence peroxisome function.

5. Concluding remarks and future prospects

Protein transport is closely linked to peroxisomal function (Fig. 3) and recent exciting reports have demonstrated that peroxisomes, in addition to their well described ability to import proteins, can also specifically export both membrane and matrix proteins. Current evidence indicates that certain export events occur in response to changes in metabolic requirements within the cell, or are involved in regulating cellular events. On the other hand, other export events may represent quality control mechanisms that remove damaged proteins from peroxisomes. Furthermore, several proteins involved in the cycling receptor export process also have a hand in membrane and matrix protein export, demonstrating significantly, that their role in peroxisome function goes much deeper than previously thought.

Nevertheless, the study of peroxisomal protein export is still in its infancy and future studies aimed at identifying additional substrates of the export pathway will allow us to gauge the scope of peroxisomal protein export. In addition, as we only have a few mechanistic details on how a subset of peroxisomal proteins are exported (Figs. 1 and 2), it will be interesting to determine the full extent to which ubiquitination controls membrane and matrix protein export, including the identity of E2s, E3s and membrane dissociation factors that may be involved in the individual export events. Here is it worth noting that in plants Pex2p interacts with DSK2a and DSK2b, two redundant ubiquitin-adaptor proteins that control the targeting of ubiquitinated substrates to the proteasome [92]. Deletion of these proteins did not affect matrix protein import, suggesting that they play different roles in peroxisome function.

Additional questions that arise include the role of proteins that control the import of membrane/matrix proteins in the export process. It will be of particular interest to see whether the cycling receptor Pex5p or the docking factor Pex14p play roles in matrix protein export, since both proteins facilitate the transport of matrix proteins from the cytosol to the peroxisomal matrix [79,80].

Finally, there exists an intimate link between peroxisome function and human health [8–10]. With this in mind, a very pertinent question is to what extent could disturbances in peroxisomal protein export contribute to human disease? Furthermore, would disorders that stem from

export defects exhibit symptoms similar to classical peroxisome biogenesis disorders? Studies that focus on the mechanisms underlying the peroxisomal export process, as well as those aimed at determining the full repertoire of exported substrates, will undoubtedly provide new insights into the role of peroxisomes in human health.

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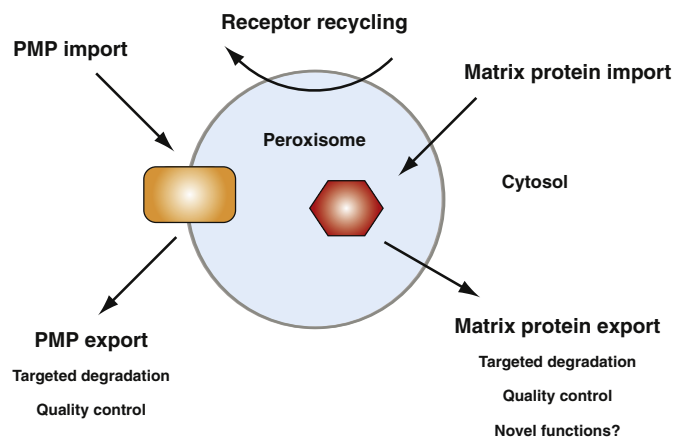


Fig. 3. The protein transport pathways of peroxisomes. Schematic representation of peroxisomal membrane (orange) and matrix (red) protein transport events, outlining the potential functions of the export pathway.

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